IN THE UNITED STATES PATENT ANC TRADEMARK OFFICE

Applicant(s):	Stavrianopoulos et al.	
Serial No.:	08/486,070	Group Art Unit: 1631
Filed:	June 7, 1995	Ex'r: Ardin H. Marschel, Ph.D.
For:	**ARRAYS AND SYSTEMS COMPRISING ARRAYS FOR GENETIC ANALYSES AND OTHER APPLICATIONS**	

60 Executive Boulevard
Farmingdale, NY 11735-4716

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DECLARATION OF DR. DOLLIE M. W. KIRTIKAR UNDER 37 C.F.R. §1.132

I, Dollie M. W. Kirtikar, hereby declare as follows:

BACKGROUND

1. I am presently Senior Scientist for Enzo Biochem, Inc., 60 Executive Boulevard, Farmingdale, New York 11735-4716, having held that position since 1997. Prior to my present position, I was Research Scientist for Enzo, having been first hired in February 1979. During the early 1990s, I held other positions within Enzo or its subsidiaries, including Production Manager and Supervisor of QA/QC. My professional experience is listed on my curriculum vitae attached as Exhibit 1.

2. In terms of my education, I received my Bachelor of Science (B.Sc.) with honors from the University of Bombay, Bombay, India in 1952, graduating with a major in microbiology and a minor in chemistry. In 1957, I received a Master of Science (M.Sc.) from the Seth C.S. Medical College, University of Bombay. In 1967, I was awarded a Doctor of Philosophy (Ph.D) from the University of Kansas. My doctoral thesis was titled "Phenotypic Transformation."

3. From 1969 to 1971, I received a visiting fellowship from the Nucleic Acid Research Foundation of Netherlands, under which I carried out research on enzymes and factors involved in DNA transcription in the Biochemistry Department, University of Netherlands, Croningen, Netherlands. From 1971 to 1977, I was a research associate in the Biochemistry Department, Case Western Reserve University School of Medicine, Cleveland, Ohio. At Case Western, I conducted research on enzymes involved in DNA repair following treatment with cancer-causing physical and chemical agents. From 1977 to 1978, I was a research associate in the Radiology Department, Stanford University Medical Center, Stanford, California, where I conducted research on DNA-repair deficient bacteria. My education and research experience are listed on my CV (Exhibit 1).

4. During my education and research spanning the years 1961 to 1978, I held a number of teaching positions which are listed on my CV (Exhibit 1).

5. I am the author of several scientific publications and the inventor named on several U.S. and foreign patents and patent applications. My scientific publications are listed on my CV (Exhibit 1). Representative issued U.S. patents are listed on my CV (Exhibit 1). I am also a co-inventor on the above-identified U.S. Patent Application Serial No. 08/486,070, filed on June 7, 1995 (hereinafter "the '070 application"). I am familiar with the specification for the '070 application that was filed on June 7, 1995. I have also read the Declaration of Dr. Jannis G. Stavrianopoulos, who is one of my co-inventors on the '070 application.[1] I have additionally read the Office Action mailed on July 2, 2003.

6. As a co-inventor, I am making this Declaration on behalf of and at the request of the assignee.

[1] I understand that Dr. Stavrianopoulos's Declarati n was submitted to the Unit d States Patent and Trademark Office in Applicants' June 17, 2002 Supplemental Amendment T [Their] April 10, 2002 Amendment Under 37 C.F.R. §1.116.

EXPERIMENTS UNDERLYING THE '070 APPLICATION

7. In connection with the present invention, my co-inventors and I discovered that nucleic acids could be fixed to non-porous solid supports and remain available for hybridization and detection. In our research and experiments that led to the present invention and the filing of the first application in January 1983, my co-inventors and I investigated different support materials, shapes, surfaces and treatments with respect to fixing nucleic acids to supports in hybridizable form. In at least two separate instances, we constructed an array of different nucleic acids fixed or immobilized to two different kinds of solid supports. Among the different solid supports I investigated prior to 1983 are those listed below in chronological order (most recent at the bottom). Copies of pages from my laboratory notebook are also included herewith as Exhibits 2-9.

SUPPORT/SURFACE	TITLE OF LABORATORY NOTEBOOK PAGE(S)	EXHIBIT NO.
[flat] microscope slides with slots (preprinted slides)[2]	Detection of glucosylated DNA with fluorescent Con-A on slides (microscope slides with slots)	2
glass tubes	Con-A binding to glucosylated DNA treated glass tubes -- Binding of DNA to glass	3
glass tubes	Concentration curve for DNA binding to activated glass tubes (Con A binding to DNA on the glass surface)	4
plastic wells	DNA binding to activated surfaces -- plastic wells treated with epoxy-glue	5
plastic plates	Activated plastic plates -- Detection of glycosylated DNA by con A -- alkaline phosphatase	6
glass tubes	DNA binding to activated glass surface	7
glass fiber filters	Preparation of glass fibre filter	8
glass tubes/slides	Preparation of Silanized glass	9

[2] My laboratory notebook refers to "microscope slides with slots." In common parlance, "slots" may suggest rectangular channels, troughs or indentations. In the context of my experiments, h wever, "slots" refers t the flat circles that are preprinted onto flat microscope slides. It is known in th art that 'slots' is a synonym for the circles on preprinted slides, also known as cytology slides.

8. Since the focus of our investigations was attachment of nucleic acids to ostensibly inert materials, such as glass and plastic, I focused upon surface treatments for glass and plastic that would permit such binding. Because the shape of these materials is irrelevant to their surface chemistry, I used a variety of differently shaped supports to carry out these experiments, including microscope slides, glass fibers, test tubes, microtitre plates and wells.

FLAT MICROSCOPE SLIDES WERE USED IN OUR EXPERIMENTS

9. As indicated above and as shown in my laboratory notebook pages (Exhibits 2 and 9), on at least two separate occasions I fixed nucleic acids to flat microscope slides.

ARRAYS OF DIFFERENT NUCLEIC ACIDS WERE CONSTRUCTED ON SEPARATE OCCASIONS WITH DIFFERENT SOLID SUPPORTS

10. As shown in my laboratory notebook pages (Exhibits 2 and 8), I constructed two nucleic acid arrays[3], one using preprinted microscope slides and the other using glass fiber filters. See the second page of both Exhibits 2 and 8.

A. In the first array (Exhibit 2, second page), T4 and λ DNA were spotted on twelve locations on the same flat microscope glass slide. The flat microscope slide was a preprinted glass slide, also called a cytology slide.

B. In the second array (Exhibit 8, second page), T4 and λ DNA were each spotted three times on the same glass fiber filter. In fact, two such arrays in the form of glass fiber filters were taped to my original laboratory notebook page

[3] This is true under a restrictive definition of "nucleic acid array" as meaning a plurality of various nucleic acids arranged on a solid support. Under the plain definition – a plurality of (various or identical) nucleic acids arranged on a solid support – more of my experiments could be charact rized as involving construction of nucleic acid arrays.

(Exhibit 8, second page) at the time the experiment was performed and both arrays are still taped there.

MY EXPERIMENTAL WORK IS IN THE '070 APPLICATION AS FILED

11. The '070 application as originally filed is completely consistent with the experiments discussed above in that it reasonably conveys that (1) the invention resided in the attachment of nucleic acids to the surface of a non-porous solid support; and that (2) the shape of that surface of the non-porous solid support was irrelevant to the invention; and, therefore, (3) that a person skilled in the art at the time the application was filed would not have viewed arrays as being limited to those including wells or depressions because of the irrelevance of the shape of the surface or solid support to which the nucleic acid was bound.

The first conclusion is manifest from page 10 of the '070 application, stating that:

> In accordance with the practice of this invention, analytes in a biological sample are . . . directly fixed to a suitable solid support. . . . It is preferred that the solid support to which the analyte is fixed be non-porous.

The second conclusion, that the shape of the solid support is irrelevant, is likewise made manifest on page 10, which states that:

> [I]t is preferred that the solid support to which the analyte is fixed be . . . glass, or alternatively, plastic, polystyrene, polyethylene, dextran, polypropylene, and the like.

It will be appreciated that the above cited passage *makes no reference to the shape of the solid support*. Certainly, had the shape been critical, it would have been specified. Indeed, only later in the specification at page 13, as *a preferred embodiment*, solid supports with wells or depressions are cited:

> Yet ***another*** aspect of the method of the present invention involves generating the soluble signal . . . in a device capable of transmitting light therethrough for the detection of the signal by spectrophotometric techniques. . . . ***Examples*** of the devices useful in the spectrophotometric analysis of the signal included conventional apparatus employed in diagnositic laboratories, i.e., plastic or glass wells, tubes, cuvettes or arrangements of wells, tubes or cuvettes.

Accordingly, point 3 is clearly conveyed, that a person skilled in the art was told by the '070 application that the invention resided in attaching nucleic acids to a solid support without any reference to the shape of that solid support; and that solid supports with wells or depressions were but a preferred embodiment, THEREFORE, the '070 application conveyed that an array as disclosed need not be limited to one with wells or depressions.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Oct. 28th 2003
Date

Dollie Kirtikar
Dollie M. W. Kirtikar, Ph.D.

* * * * * * *

Dollie M.W.Kirtikar
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Fresh Meadow, NY 11373
(415) 327-3442

EDUCATION:

Bachelor of Science B.Sc.	April 1952 with Honors. Microbiology as Major; Chemistry as minor. St.Xavier's College, Bombay,India. University of Bombay.
Master of Science M.Sc.	February 1957 (three years with research Microbiology as major; Biochemistry as minor. Seth G.S.Medical College, Eombay, India. University of Bombay. Thesis: Typing of Salmonella Paratyphi A Strains For All Over The World
Doctor of Philosophy Ph.D.	June 1967. University of Kansas. Kansas (U.S.) Title of Doctorial Thesis: Phenotypic Transformation

RESEARCH EXPERIENCE:

March 1969 to March 1971	Visiting fellowship from Nucleic Acid Research Foundation of Nederlands Biochemistry Department, University of Nederlands at Groningen, Nederlands Molecular biology with emphasis on enzyme, and factors involved in DNA transcription
May 1971 to September 1977	Research Associate at Biochemistry Department, Case Western Reserve University School of Medicine, Cleveland, OH 44106 Molecular biology with emphasis on enzyme involved in DNA repair after treatment with physical and chemical agents known to cause cancer.
October 1977 to June 1978	Research Associate at Radiology Department Stanford University Medical Center, Stanford, CA 94305 Molecular biology and genetics with emphasis on DNA-repair deficient bacteria
February 1979 to February 1997	Research scientist at Enzo Biochem, Inc. and its subsidiary, Enzo Life Sciences, Inc. (formerly Enzo Diagnostics, Inc.)

	Also held position as Production Manager and Supervisor of QA/QC
February 1997 to Present	Senior Scientist at Enzo Life Sciences, Inc. (formerly Enzo Diagnostics, Inc.)

TEACHING EXPERIENCE:

1961-1963 Teaching Assistantship at Microbiology Department, University School of Kansas Medical Center, Kansas City, Kansas.

General laboratory assistance in various courses offered to medical and graduate students. Preparation of course material, supervision of students conducting experiments, assistance in lab experiments, conferences with students, etc.

1965 Laboratory Instructor in Department of Pathology and Bacteriology at Topiwalla National Medical College, Bomoay, India.

Supervision of clinical laboratory staff.
Training of research personnel such as technicians, graduate students. Conferences with students etc. on particular research projects. .

1969-1971 Biochemistry Department, University of Nederlands at Groningen, (March) Nederlands.

Training of graduate students and technicians. Conferences and discussions with graduate students on particular research project

1972-1977 Biochemistry Department, Case Western Reserve University School of Medicine, Cleveland, OH 44106

General supervision of laboratory personnel; training and supervision of technicians, graduate and college students; iniation of new personnel in the laboratory group in various techniques. Conferences and discussions and suggestions to various personnel about specialized techniques in molecular biology.

Jan.1978 to June 1978 Radiology Department,Stanford University Medical Center, Stanford, CA 94305

Training and supervision of one technician in molecular genetics with particular emphasis on mapping of bacterial mutants. Preparation of phage lysate, transduction and conjugation expts, construction of strains etc.

PUBLICATIONS:

1. Mridula W. Kirtikar, Typing of Salmonella Paratyphi A Strains For All Over The World. *J. Post Graduate Medicine of India* (1957)

2. Mridula W. Kirtika, D.D. Banker, and N.W. Purandare, Bacteriophage Typing of 371 Strains of Salmonella Paratyphi A. *J. Post-Grad. Med., Vol. VI, 6* (1960)

3. N.Veeraraghavan and M.W. Kirtikar, Biologicial Characteristics of Influenza virus Strains Isolated at the Government of India Influenza Centre, Coonoor, during 1950-69, *Bull.World Hlth.Org., 24, 687* (1961)

4. N.Veeraraghavan, M.W. Kirtikar and T. Sreevalsan, Studies on the Cultivation of Influenza Virus in vitro. *Bull. World Hlth. Org., 24, 711* (1961)

5. Mridula W. Kirtikar, Phenotypic Transformation. *Dissertation Abs Intl., 28 (7), 2708* (1967)

6. Mridula W. Kirtikar and Jacob D. Duerksen, A Penicillinase-Specific Ribonucleic Acid Component from Bacillus Cereus. I. Ribonucleic Acid Extraction and Definition of the in Vivo Test System. *Biochemistry, 7, 1172* (1968)

7. Mridula W. Kirtikar and Jacob D. Duerksen, A Pencillinase-Specific Ribonucleic Acid Component from Bacillus Cereus. II. Partial Characterization of the Active Component. *Biochemistry, 7, 1183* (1968)

8. Dollie M. W. Kirtikar and Akira Kaji, Stimulation of Phage Ribonucleic Acid-dependent Incorporation of Amino Acids by 5 S Ribonucleic Acid. *J.Biol.Chem., 243, 5345* (1968)

9. Shelk-Mumtaz Hadi, D. M. Kirtikar, and D.A. Goldthwait, (Endonuclease II of Escherichia coli. Degradation of Double- and Single-Stranded Deoxyribonucleic Acid. *Biochemistry, 12, 2747* (1973)

10. D.M. Kirtikar and D.A. Goldthwait, The Enzymatic Release of O^6-methylguanine and 3-methyladenine from DNA Reacted with the Carcinogen N-methyl-N-nitrosourea. *Proc. Nat. Acad. Sci., 71, 2022* (1974)

11. D.A. Goldthwait, D.M. Kirtikar, S.M.Hadi, and E.C. Friedberg, Molecular Mechanisms for repair of DNA, Part A. *Plenum Publishing Company, p.191-196* (1975)

12. D.M. Kirtikar, J. Slaughter and D.A. Goldthwait, Endonuclease II of Escherichia coli: Degradation of γ-Irradiated DNA. *Biochemistry, 14, 1235* (1975)

13. Dollie M. Kirtikar, Anthony Dipple and David A. Goldthwait, Endonuclease II of Escherichia coli: DNA Reacted with 7-Bromomethyl-12-methylbenz[a]anthracene as a Substrate. *Biochemistry, 14, p.5548* (1975)

14. D.M. Kirtikar, J.P. Kuebler, A. Dipple, and D.A. Goldthwait, Endonuclease II of Escherichia coli and Related Enzymes, Fundamentals in Cancer Prevention: 6th Int. Symp. of the Princess Takamatsu Cancer Research Fund. *University of Tokyo Press, p.349* (1975)

15. D.M. Kirtikar, J.P.Kuebler, A. Dipple, and D.A. Goldthwait, Enzymes Involved in Repair of DNA Damaged By Chemical Carcinogens and γ-Irradiation. *Cancer Enzymology (eds. J. Schultz and F.E. Ahmed, Eighth Miami Winter Symposium, Academic Press, p.139* (1976)

16. Dollie M. Kirtikar and David A. Goldthwait, Endonuclease II and the Apurinic Acid Endonuclease of E. Coli. *Fed. Proc., 35, 1589* (1976)

PATENTS:

U.S.

- 5,241,060
- 5,260,433
- 4,994,373

* * * * * * *

Detection of glycosylated DNA with fluorescent con-A on slides. 6/1/82.

Norman Keller 6/1/82

Microscope slides with slots
Treated for 5' with 0.1 % HCL
Washed with water 5 X
Treated with subbing solution
0.1 % $CrK(SO_4)_2$
1 % gelatin
for 5 minutes
Air dried.

Apply DNA 20 λ
Air Dry
Wash with 70 % EtOH 20'
followed by 95 % EtOH 20'
Air Dry

Apply con A 20 λ in NaCl - Mg^{++} 10 mM eac

~~Air t~~ Keep in HUMID ~~Hubrid~~ chamber
for 30'
Rinse with PBS
keep in PBS ~~7~~ minutes

20λ	~~5λ~~	10λ	15λ	20λ		DNA
O	O	O	O	O	O	

DNA (T4 or λ)	20λ	5λ	10λ	15λ	20λ	—
con A	—	20λ	20λ	20λ	20λ	20λ

all positive*	O	O	O	O	O	O	T4 DNA
all negative	O	O	O	O	O	O	λ DNA

Positive

* gave a characteristic fluorescent green color

5
5
0.5

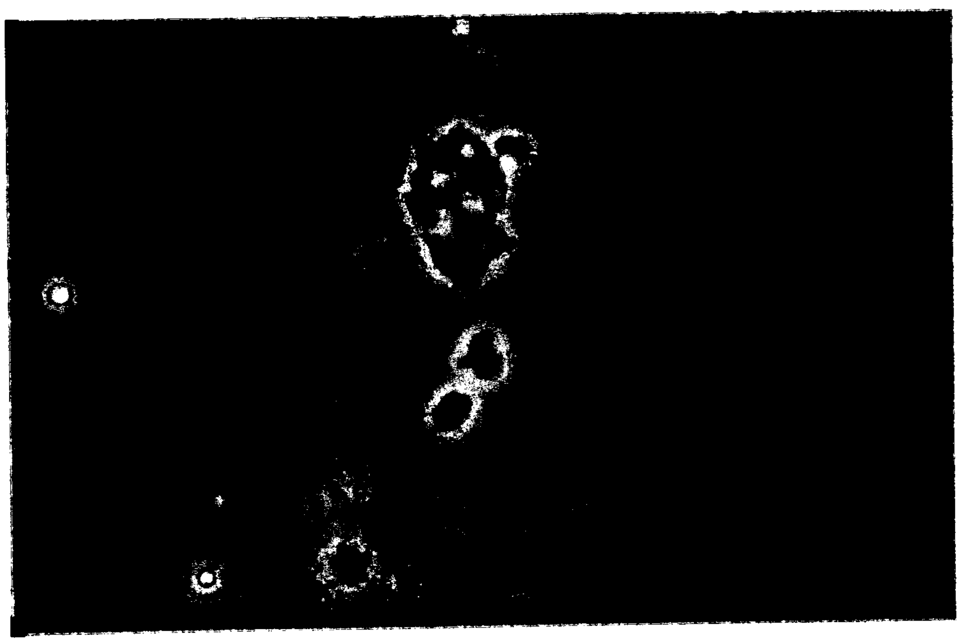
Wash slides 0.1 N HCl, 5 min
Rinse d H_2O 5X
1% gel, 0.1% Cr K $(SO_4)_2$ 5 min / dry

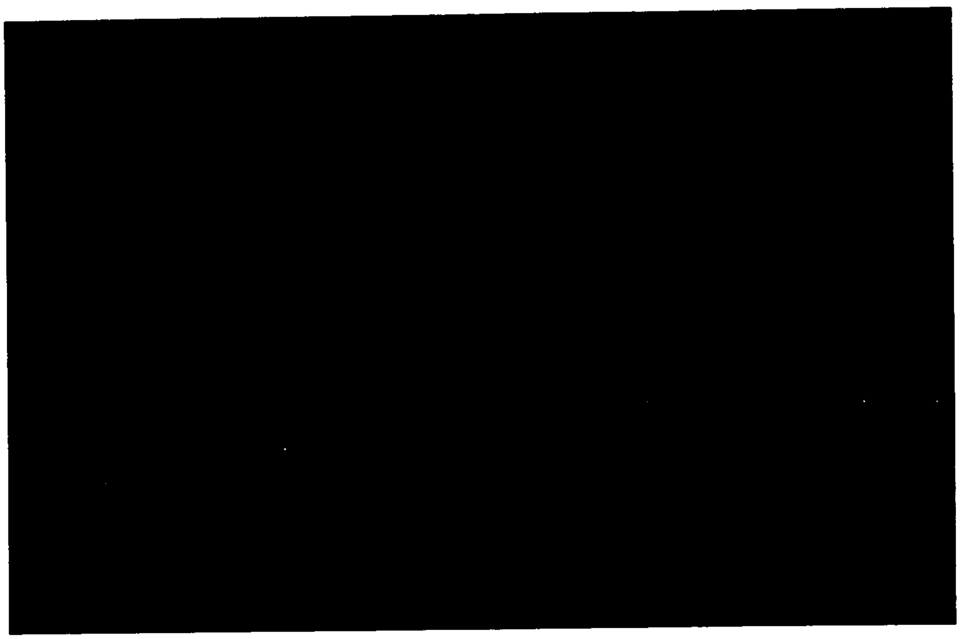
Apply Chromosome
70% EtOH, 95%, dry
0.1 N NaOH
EtOH
95% Form 68°C, 1 hr
EtOH

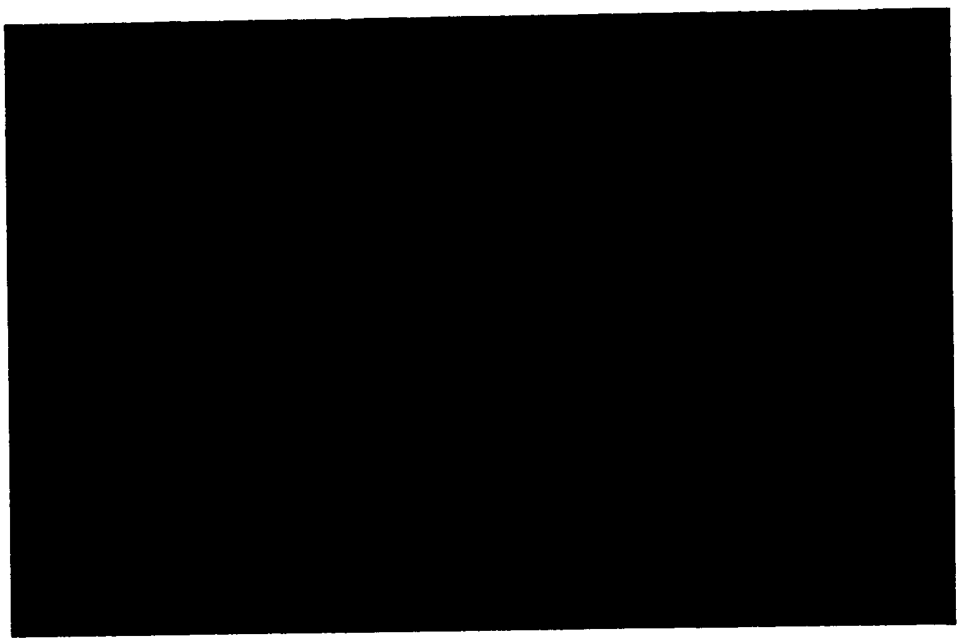
Hybridize
45% [illegible], 4x SSC, Dextran [illegible]
[illegible]°C

conA 50γ/ml — 20γ 20γ
T4DNA –

λ DNA
ConA – 20







5

6.13.82

1 Con A binding to glucosylated DNA

Norman Kelly 6/13/82

Treated glass Tubes

	1	2	3	4	5	6	7	8	9
T4 DNA	100λ	–	100λ	–	100λ	–	–	–	–
CT DNA	–	100λ	–	100λ	–	100λ	–	–	–
Fluorescent Con A	–	–	100λ	100λ	100λ	100λ	100λ	100λ	–
carrot phosphatase	–	–	–	–	+	+	+	–	~~+~~

After each addition tubes incubated at RT for 15-30 minutes

Washed repeatedly c̄ PBS 100λ aliquots 20 times.

Tubes 1 and 2 stained with 100λ of 1 µg/ml EtBr in PBS 30' in the dark. at room temp

To tubes 3, 4 and 8 added con A ~~=~~ fluorescent ~~@~~ 5 mg/ml in NaCl Mg^{++} made up to 10 mM.

Left at room temp for 1 hour

Washed repeatedly c̄ PBS.

Binding of DNA to glass

Checked under U.V lamp after Ethidium bromide staining of tubes 1+2 and Fluorescent Con A interaction with tubes 3, 4 + 8

1 2 3 4 8

Tubes 1 & 2 gave a red fluorescence characteristic of Ethidium bromide and tube 3 gave a green fluorescence. ~~[illegible]~~ Tubes 4 and 8 gave an ~~[illegible]~~ visible green fluorescence.

To tubes 5, 6, 7 added unlabelled con A

5 mg/ml in PB containing NaCL, Mg^{++}, ~~Ca^{+}~~

left at room Temp for 60 minutes

washed repeatedly with PBS 20 times.

washed with Imidazole-HCL 5X 0.2M pH 6.5

Added acid phosphatase 1/10 diluted 10 λ 2 units

Added diluent containing BSA 100λ

left at RT for 30'

Washed repeatedly with Imidazole-HCL 20 times.

Added 20λ substrate
80λ buffer

left at RT

color intense yellow in tube #5

immediately, Added 1.0 ml bicarbonate

checked at [illegible]

A_{300} in ~~50~~ 5' on ~~/~~ 1:1 diluted samples.

# Tube	A_{300} vs H_2O blank
5	0.498
6	0.065
7	0.055
9	0.011

λ DNA nick-translated c̄ malto-triose substituted UTP was attached to the glass similarly. Con A was added, washed off.

detection of conA binding to DNA was carried out using acid phosphatase in the same manner as for T4 DNA.

Tube #		A_{300}
10	λ DNA control nick translated c̄ TTP	0.009
11	λ DNA test (nick translated with maltotriose dUTP)	0.368
12	con A + acid phosphatase	0.012
13	acid phosphatase alone	0.008

Norman Keller 6/17/82 3.

6.17.82

Concentration curve for
DNA binding to activated glass Tubes.

T4 DNA - . ^{3}H T4 DNA nick-translated using
3.29 micrograms/ul ^{3}HdATP

λ DNA - ^{3}H λ DNA nick translated using
3.615 micrograms/ul ^{3}HdATP

			^{3}H cpm	
5λ	^{3}H T4 DNA	16.45 ng	7809 6630	7220

approx. 439 cpm/nanogram

5λ	^{3}H λ DNA	18.075 ng	5044 4354	4699

approx. 260 cpm/nanogram

^{3}H 6.17.82

T4 5λ 16.45ng

1 01
001.00
003.00
007809.00
1 02
001.00
003.00
006630.00

^{3}H λ DNA 6.17.82
5λ 18.075ng

1 05
001.00
003.00
005044.00
1 06
001.00
003.00
004354.00

4699

^3H T4 DNA 10λ diluted to 1.0 ml.

32.9 ng/ml.

	DNA			
1. A	10λ	+ 490λ buffer	— 658	picograms/ml
2. B	20λ	+ 480λ buffer	— 1.316	nanograms/ml
3. C	40λ	+ 440λ buffer	— 2.632	nanograms/ml
4. D	50λ	+ 450λ buffer	— 3,290	nanograms/ml
5. E	100λ	+ 400λ buffer	— 6.58	nanograms/ml
6. F	200λ	+ 300λ buffer	— 13.16	nanograms/ml
7. G	500λ	+ 00	— 32.9	nanograms/ml.

^3HT4 DNA 25λ diluted to 1.0 ml

82.25 ng/ml.

8. H 500λ + 0 buffer 82.25 ng/ml.

3HT4 DNA 50λ diluted to 1.0 ml.

164.5 ng/ml.

9. I 500λ + 0 buffer 164.5 ng/ml.

3H T4 DNA 100λ diluted to 1.0 ml.

10. J. 500λ + 0 buffer. 329.0 ng/ml.

100 λ of each solution checked for radioactivity after dilution c̄ 200 λ of 5 mM TRIS 7.4

100 λ of each solution placed in
A nonactivated glass Tubes
B activated glass Tubes.

After 5' at R.T. solutions removed carefully
tubes washed 2 x c̄ 100 λ portions of PBS.Mg^{++}

~~All com~~ washes and originals combined and counted.

^3H epm

	Original - Bkg(3.5)			Non-Activated - Bkg(3.5)		Activated - Bkg(3.5)	
1	65.8 pgm	23	100%	23	100%	16	69.56%
2	131.6 pgm	32	100%	24	75%	27	84.38
3	263.2 pgm	48	100%	49	100%	32	66
4	329.0 pgm	65	100%	70	107.6%	39	60
5	658 pgm	106	100%	111	104.7%	97	91.5
6	1.316 ngm	245	100%	255	104%	167	68.16
7	3.29 ngm	527	100%	569	108%	468	82.25
8	8.225 ngm	1337	100%	1348	100.8%	979	73
9	16.45 ngm	2387	100%	2428	101.7%	2089	87.5
10	32.9 ngm	4053	100%	3883	95.8%	3060	75.5

Dollie 6/21/82

Dollie 6/21/82

10'

34

7'

5878

9

9

a

1

2' Remained on Activated glass surface

1.	20 picograms.
2.	20.56
3.	89.49
4.	131.6
5.	55.93
6.	419.00
7.	584.00
8.	2220.75
9.	2052.96
10	8060.5

10' picograms on glass surface

1	28.95
2.	17.10
3.	131.6
4.	118.44
5.	184
6.	355
7.	428
8	2303
9.	2139
10.	8554

Con A binding to DNA on the glass surface

Con A 100 μg/ml in PBS
1 μg/ml "
10 ng/ml "

	DNA on Surface	Con A	R.T. 60'
1	~~20 pcg~~ 28.95	100 pcgm	
2.	~~20.56~~ 17.10	100 pcgm.	
3.	~~89.49~~ 131.6	100 ngm.	
4.	~~131.6~~ 118.44	100 ngm.	
5.	~~55.93~~ 184	100 ngm.	
6.	~~419~~ 355	10 μgm.	
7.	~~584~~ 428	10 "	
8.	~~2220~~ 2303	10 "	
9.	~~2052~~ 2139	10 "	
10.	~~8060~~ 8554	10 "	

6.30.82.

Con A removed and the tubes washed
5 times with PBS. 100 λ
2 times with Imidazole buffer pH 6.5
Alkaline phosphatase 10 mg/ml diluted
to 0.5 mg/ml in Tris pH 7.4

100 λ added to each tube.
Incubated at RT 30 minutes.
Removed and washed with Imidazole buffer pH 6
3 times 100 λ

Added 20 microliters of substrate 0.1 M
80 microliters of Tris pH 7.4 ~~5mM~~ 2 M
Incubated at RT.
Within 10 min. the color developes.
After 30 min. at RT.
Diluted 1/10 in ~~1~~ M Tris pH 7.4
and read at 410 vs. substrate blank.

A_{410}
X10. ~~X10~~

DNA on surface.

1	28.95 pcg.	0.385
2	17.10	0.296
3.	131.6	0.6
4.	118.44	0.58
5.	184	0.6
6.	355	0.67
7.	428	0.43
8.	2303	~~1~~ 0.68
9.	2139	~~1~~ 0.68
10.	8559	~~1~~ 0.69

untreated 11. Enzyme + Substrate blank. 0.71
untreated 12. Enzyme + substrate ~~blank~~ + con A 0.705
treated 13 Enzyme + Substrate + con A blank. 006
treated 14 Enzyme + Substrate blank. 003

6.30.82

DNA binding to activated surfaces.

plastic wells treated with epoxy-glu.
5λ and 10λ
air-dried.

^3H T_4 nick translated DNA 32.9 ug/ul
diluted to contain

A 0.658 ug/ul. in 5 mM TRis 7.4.
B 1.316 "
C 2.632 "
D 3.290 "
E 6.580 "
F 13.160 "
G 32.90 "

50 microliter added per well.
after 10' at RT.
samples removed
wells washed with PBS pH 6.5 twice.
50 microliter aliquots.
all washes combined and counted
~~Samples~~

50 λ of 1 Mg/ul con A in PBS.Mg++
applied to each well.
Left at RT. for 30'
washed ē imidazole buffer twice

Applied 50 microliters of alkaline phosphatase
0.5 mg/ml
After 30 minutes at RT.
washed ē Tris containing 0.15 M KCl
3 times.
finally with Tris alone.
Applied 0.1 M substrate 5 λ in
45 λ Tris 7.4 2.0 M.

Immediate color developed. but
the enzyme by itself also sticks.

6.20.82

1	2	3	4	5	6	7	8	9	
1A	Epoxy Glu 5λ								
DNA Applied.	32.9	65.8	131.6	164.5	329	658	1645		
	← picograms. →								
	14	29.6	59.2	74	148	296	740.3		
	← 3H cpm. →								
DNA not bound.	13.5	13.5	24	49	73	165.5	393.5		
	← 3H cpm →								
DNA on surface	0.5	16.1	35.2	25	75	130.5	346.8		
	← 3H cpm →								
	1.11	35.74	78.15	55.5	166.5	289.7	769.9		
	← picograms →								
Con A	50	50	50	50	50	50	50	50	
	← nanograms. →								
Alk. Phosp.	25	25	25	25	25	25	25	25	25
	← micrograms. →								

6.30.82

1	2	3	4	5	6	7	8	9	
B 10 λ	Epoxy	Glu.							
DNA	32.9	65.8	131.6	164.5	329	658	1645		
			picogram.						
³H hepar.	14	29.6	59.2	74	148	296	740.3		
			picograms.						
³H hepar. not bound	5.50	13	26.5	38.5	95	185	418.5		
- BI 15	8.5	16.6	32.7	35.5	53	111	321.8		
DNA bound to surface	18.9	36.9	72.59	78.81	117.7	246.4	714.4		
			picograms						
Con A	50	50	50	50	50	50	50	50	
			nanograms.						
Alk. phosp.	25	25	25	25	25	25	25	25	25
			micrograms.						
Substrate									

Dollie
6/29/82

1 19[illegible]
000083.50
1 29
002.00
015.00
000110.50
1 30
002.00
015.00
000181.00
1 31
002.00
010.00
000201.00
1 32
002.00
007.00
0[illegible]
1 33
002.[illegible]
[illegible]7.[illegible]
[illegible]34.[illegible]
1 3[illegible]
[illegible]

Norman Kelker
7/13/82

Activated plastic plates 7.13.82

Detection of glycosylated DNA Norman Kelker

by con A - alkaline phosphatase 7/13/82

^3H T_4 DNA nick translated 3.29 µg/ml
diluted in PBS buffer to
32.9 nanograms/ml

Con A 10 mg/ml in 2.0 M NaCl
50 µg in 5 lambda.

alkaline phosphatase 0.5 mg/ml in
5 mM TRIS pH 7.4

4-Nitrophenyl phosphate 1 mg/ml in
1 M TRIS pH 7.4

BSA 20 mg/ml phosphatase free
from Janos.

1. DNA 50 λ RT 10'
Rinse 3 x ē 100 λ PBS
Count
2. Con A 5 λ (50 µg) in PBS. Mg^{++}
Total 50 λ
RT 30'
3 Rinse 3 x ē PBS Mg^{++} 100 λ each rinse
2 x ē TRIS. HCL 100 λ each rinse
4 BSA 20 mg/ml 50 λ
37° 2 hours.
5. Rinse ē TRIS. HCL 5 mM 2 x
6. Alkaline phosphatase 50 λ 25 µg
30' RT.

Norman Keller
7/13/82

7. Rinse 5 x c 100 λ of 0.3 M NaCl
8. Substrate 2 mg/ml ~~10~~ x ~~20~~ ug [redacted]
Incubate at 37°

	A	B	C	D	E	F	G	H
DNA	+	+	+	+	−	−	−	−
ConA	+	+	−	−	+	+	−	−
BSA	+	+	+	+	+	+	+	+
AP	+	−	+	−	+	−	+	−
NaCl	+	+	+	+	+	+	+	+
Substrate	+	+	+	+	+	+	+	+

7/13/82

1 88
005.00
13kg.
000 17.20
1 89
original
1 90
well ①
1 91
well ②
1 92
well ③
1 93
well ④

	A_{410}
A	0.149
B	000
C	0.015
D	0.012
E	0.099
F	0.058
G	0.053
H	0.000

MICROELISA

1	A	>	1.007
1	B		0.002
1	C	>	0.749
1	D		0.006
1	E	>	0.727
1	F		0.025
1	G		0.149
1	H		0.000
2	A	>	1.379
2	B		0.001
2	C		OVER
2	D		0.064
2	E		OVER
2	F		0.026
2	G		0.015
2	H		0.012
3	A		OVER
3	B	>	0.304
3	C		OVER
3	D	>	0.343
3	E		OVER
3	F	>	0.401
3	G		0.099
3	H		-0.001
4	A		OVER
4	B		0.002
4	C	>	0.381
4	D		0.012
4	E	>	0.830
4	F		0.031

[redacted]

DNA binding to activated glass surface 8.2.82.

T_4 DNA 4 µg/ml

diluted to contain

A 1000 ng/ml

B 100 ng/ml

C 10 ng/ml

D 1 ng/ml

E 0.4 ng/ml

100 λ of each solution in duplicates
in activated glass tubes

10′ at RT.

Remove carefully.

Rinse tubes 3 x c̄ 100 λ of 2 x SSC

100 λ of con A added 0.1 mg/ml in PBS. Mg^{++}

F con A

G PBS. Mg^{++}

left at RT for 60′ 11.47 am.

Rinse 3 x c̄ 100 λ of 2 x SSC. Incubate

To set 1 added 100 λ PBS. Mg^{++} RT 60′

set 2 added 100 λ 1% Ficoll 400

Wash 3 x c̄ 2 x SSC

Add 100 λ PBS·Mg++ to set I
100 λ Con A 0.1 ug/ml in PBS·Mg to set II

60' at RT.

Wash 3 x c̄ 2 x SSC
3 x c̄ 0.1 % BSA in 2 x SSC

Add 100 λ 1 % BSA in 2 x SSC to
all tubes.

Cover c̄ parafilm
Keep at 4° o/n.

8/3/82

Rinse 3 x c̄ 2 x SSC containing 0.1 % BSA

Add 10 λ of 1/5 diluted acid phosphatase
40 λ of diluent containing 0.1 % BSA

Incubate at RT for 30'

Remove

Rinse 5 x c̄ 0.3 M NaCl

Add 20 λ of 0.1 M substrate
80 λ of 0.2 M Imidazole buffer (6.5)

Incubate at 37° for one hour.

~~Dilute~~
Stop Rx $\bar{c}$ 2.0 ml 5% $NaHCO_3$.

Check OD at A_{410}.

1	2 DNA	3 ConA	4 Ficol	5 ConA	6 Acid P.	7	A410	9
A	50 ng	+	–	–	+		0.285	x20
A2	50 ng	+	+	+	+		0.35	x20
B1	5 ng	+	—	–	+		0.175	x20
B2	5 ng	+	+	+	+		0.23	x 20
C1	500 pcg	+	—	–	+		0.06	x 20
C2	500 pcg	+	+	+	+		0.12	x 20
D1	50 pcg	+	–	–	+		0.11	x 5
D2	50 pcg	+	+	+	+		0.22	x 5
E1	20 pcg	+	–	–	+		0.144	x 2
E2	20 pcg	+	+	+	+		0.208	x 2
F1	—	—	—	—	+		0.006	
F2	—	+	+	+	+		0.007	
G1	—	—	—	—	+		0.006	
G2	—	—	+	—	+		0.005	
Control	—	—	—	—	+		0.35	x20

Preparation of glass fibre filters.

EGF Filters. soaked in 5% Nitric Acid For 4 days.

Washed c̄ H_2O

DRIED 24 hours at 100°

10% Organosilane prepared pH 3.11 adjusted c̄ 6 N HCL [and end point c̄ diluted HCL]

Equilibrated to 75° in brown bottle.

Washed filters dropped one by one in solution.

Swirled gently once so all the filters were evenly dispersed.

Incubation at 75° for 2 hrs and 45 minutes.

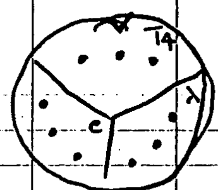
Filters removed liquid sucked off on ~~vaccuum pum~~ filtration apparatus.

Filters washed once with distilled water

DRIED o/n at 100°

8/28/82.

Two Filters used



T_4 DNA 1λ per spot $\boxed{0.2 \text{ ug/ml}}$

λ DNA 1λ per spot $\boxed{0.2 \text{ ug/ml}}$

PBS·Mg^{++} 1λ per spot

AIR DRY.

Wash c̄ PBS·Mg^{++} on Filtration apparatus.

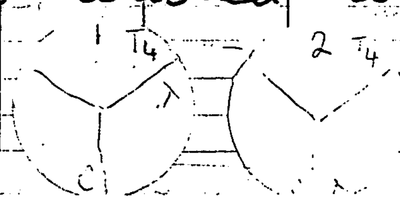
Filter # 1 stained c̄ EtBR.

Filter # 2 soaked in FITC-labelled con A

0.5 ug/ml 100λ per filter.

excess fluid removed by filtration

Filter washed with PBS·Mg^{++} buffer.



9/1/82

2	3 ^{3}HT4	4–5 CT DNA 2.5 ug/ul	6–7 1X SSC	8 3.0 M NaOH	9 2.0 M Am Ac
0	20λ	0	160λ	20λ	200λ
1	"	10λ	150λ	20λ	200λ
2	"	20λ	140λ	20λ	200λ
3	"	40λ	120λ	20λ	200λ
4	"	80λ	80λ	20λ	200λ
5	"	160λ	0	20λ	200λ

10' at RT

2 Sets.

One set filtered thru activated glass filters

Control set filtered thru EGF untreated

Radioactivity checked.

1 23
002.00
001.50
018086.00
1 24
002.00
000.00
000019.00
1 25
002.00
007.00
000647.50
1 26
002.00
015.00
000163.50
1 27
002.00
010.00
000223.50
1 28
002.00
015.00
000124.50
1 29
002.00
015.00
000117.00
1 30
002.00
015.00
000155.00
1 31
002.00
000.00
000018.00
1 32
002.00
015.00
000115.00
1 33
002.00
020.00
000053.00
1 34
002.00
020.00
000054.00
1 35
002.00
020.00
000080.50
1 36
002.00
020.00
000055.00
1 37
002.00
000.00
000032.00

Treated Filters.

^3H cpm. T4 DNA	Competition $\bar{c}$ unlabelled CT DNA μg.	Counts on Filter	% Inhibition	Capa[city] of Fi[lter]
18086	0	18035		
	25	18063		
	50	18023		
	100	18024		
	200	18017.		
	400	18035		

Untreated Filters

^3H cpm. T4 DNA	Competition $\bar{c}$ unlabelled CT DNA μg.	Counts on Filter	% Inhibition	Capa[city] of Fi[lter]
18086	0	18155		
	25	18018		
	50	17115		
	100	8053		
	200	3723		
	400	2413		

Preparation of Silanized glass:

Glass Tubes (slides) are boiled for 45 min. in 5 % nitric acid solution, then washed and dried for 24 hours at 115 °C. ~~the~~ The surface area to be activated is dipped into a 10 % solution of gamma-aminopropyltriethoxysilane (Union Carbide Silicones A-1100) dissolved in distilled water and pH adjusted to 3.45 with ~~~~ normal hydrochloric acid. The suspension is placed for 2.75 hours at 75°, removed and then washed with the same amount of water ~~(same volume) twice~~ and then dried overnight at 100 °C. The resulting material has an available alkylamine.

Activated glass tubes were used to show con lectin-binding to glucosylated [glucose-substituted nick translated] DNA. Experimental data indicated that ~~although glucosylated and non g~~ although there was no difference in binding of ~~DNA~~ either glucosylated

⊕ the endpoint of the pH titration is made with .6M HCl (1/10 dilution) the solution warms up during the neutralyzation so that the pH ⊕ reads 3.2 wich corresponds to 3.45 at 25°C.

or non-glucosylated DNA to activated glass surface [range 60 picograms to 10 nanograms as detected by [^3H] radioactive DNA], fluorescent labelled con-A bound only to glucosylated DNA [DNA was counter stained with ethidium bromide]. ~~Further experiments indicated that~~ That lectin bound only to glucosylated [glucose-substituted DNA by nick translation] was further substantiated by the fact that and ~~horse~~ (...t) phosphatase ~~radish peroxidase~~ (a glycoprotein) bound ~~only~~ to con A which already was stuck to glucosylated DNA. ~~Both con A~~ Enzyme activity was ~~found~~ not washed off from these tubes. Both con A and horse raddish peroxidase do not bind to the activated glass surface.